Genotyping of *Burkholderia mallei*: Effective Subspecies Discrimination using Ribotyping and Repeat Sequences

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Bioforensics

- **<u>Bioforensics:</u>** Identification of microorganisms for the purpose of attribution. Originally for epidemiological purposes; more recently for the purpose of tracking the source of an intentional release.
  - Mostly DNA-based (specificity, stability)
  - Essentially analogous to human DNA "fingerprinting" used for paternity testing and other forensic applications
  - Also can involve physical, chemical or immunological characteristics

Burkholderia mallei Biology and Pathogenesis

- Previously known as *Pseudomonas mallei, Malleomyces mallei,* and *Actinobacillus mallei*
- Gram-negative rod and an obligate parasite. Causative agent of glanders in horses, donkeys and mules and can also infect humans, cats, dogs and many other mammals under experimental conditions.
- Hamsters or mice are the most common laboratory models
- Mortality is very high
- There is no vaccine
- Antibiotics are typically only partially effective leading to latent infections with the organism sequestered in abscesses in organs
- Glanders has disappeared from most regions of the world
- Enzootic foci in Asia and occasional human cases among veterinary and laboratory workers

Intentional B. mallei Releases – Published Accounts

- Wheelis, M., "First shots fired in biological warfare, Nature 395, p. 213, 1998
  - 1915: Dr. Anton Dilger, a German-American physician living in Washington, D.C., grew *Bacillus anthracis* and *B. mallei* cultures from seeds supplied by the Imperial German Government. Dilger gave the cultures to German agent dockworkers in Baltimore, who in turn infected some 3,000 head of livestock destined for Allied troops in Europe. Several hundred troops were infected. Similar activities in Romania, Spain, Norway and South America.

- Some other less-documented accounts:
  - Alibek, K., Handelman, S., Biohazard. 1999, New York, New York, Dell Publishing. p. 268-269. Alibek says he was told that *B. mallei* had been used by Soviets on mujaheddin in remote regions of Afghanistan in 1982.
  - During World War I glanders was believed to have been used to infect large numbers of Russian horses and mules on the Eastern Front.

Recent B. mallei Work: Antibiotic Resistance

- Ageeva et al. (1989)* transferred pTH10a from *E. coli* KS707 into six wild strains of *B. mallei* The pTH10 plasmid was stably maintained in *B. mallei* even without the selective pressure of antibiotics. Transconjugants simultaneously inherited tetracycline, kanamycin and ampicillin resistance genes. The conjugal function of the plasmid was also retained, permitting transfer of the antibiotic resistance genes between *B. mallei* strains with the same frequency as from *E. coli* KS707 (pTH10).
  - *Ageeva, P.P., Merinova, L.K., and Peters, M.K., "Use of the Plasmid pTH10 for the Production of Donor Strains of Pseudomonas mallei.", Molekulyarnaya Genetika, Mikrobiologiya I Virusologiya No. 4, pp. 14-18 (1989)

Recent B. mallei Work: Correlation of antibiotic resistance and virulence

- **Stepanshin et al. (1994)* did a correlation of the frequency of mutations determining resistance to five different antibiotics and "the influence of the resistance mutations on the culture virulence".**

  - ***Stepanshin IuG, Manzeniuk IN, Svetoch EA, Volkovoi KI, "In Vitro Development of Fluoroquinolone Resistance in the Glanders Pathogen.", Antibiot Khimioter (1994)**

B. mallei - Recent

- ***Arjun Srinivasan, M.D., Carl N. Kraus, M.D., David DeShazer, Ph.D., Patrice M. Becker, M.D., James D. Dick, Ph.D., Lisa Spacek, M.D., John G. Bartlett, M.D., W. Russell Byrne, M.D., and David L. Thomas, M.D.*** **Glanders in a Military Research Microbiologist, New England Journal of Medicine 345:246, 2001.**

Conclusion of Recent B. mallei work

- ***B. mallei*** **is a potentially problematic organism**
  - **Disreputable history**
  - **Some recent work apparently oriented towards increasing its pathogenic potential and/or antibiotic resistance**
  - **Historically high mortality, no vaccine and relatively poor treatment options**
  - **CDC Category B Bioterrorism agent http://www.cdc.gov/**

- **Important to develop forensic ability to track the potential source of an outbreak**

Genotyping of B. mallei

- **Five *B. mallei* isolates were not discriminated by Multilocus Sequence Typing (Godoy et al., J. Clin. Microbiol. 41:2068, 2003)**

- **Ribotyping has been used with many pathogens, including the closely-related *Burkholderia pseudomallei*. It enables the genetic analysis of an organism without prior knowledge of its sequence**

- **Here, DNA was prepared from 25 *B. mallei* isolates from human and equine infections that occurred at various times around the world**

Strain Information – Diverse Origins

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Strain	Other Names	Source	Place of origin	Date	Species
GB3	Strain A, 120	Lister Institute, London then USAMRIID	U.K.	1920	
GB4	10248, Strain 6	USAMRIID	Ankara, Turkey	1950	Human
GB5	10229, Strain Budapest	USAMRIID	Pecs, Hungary	1961	
GB6	10260, Strain 11	USAMRIID	Ankara, Turkey	1949	Human
GB7	10247, Strain 12	USAMRIID	Ankara, Turkey	1960	
GB8	Strain China 7	USAMRIID			
GB9	Strain 102	Imperial Inst. Vet. Res., then USAMRIID	India	1932	Mule lung
GB10	Strain 106	Imperial Inst. Vet. Res., then USAMRIID	Inida	1932	Horse
GB12	Ivan, NCTC 10230	USAMRIID	Pecs, Hungary	1961	
Turkey 2		USDA-APHIS	Turkey		
Turkey 4		USDA-APHIS	Turkey		
Turkey 6		USDA-APHIS	Turkey		
Turkey 7		USDA-APHIS	Turkey		
Turkey 9		USDA-APHIS	Turkey		
85-503		Col. V.C. Micra, then USDA-APHIS			Equine
86-567			East India		Mule
ISU					
273	2002721273	CA Gleiser Army Med School	USA	1956	
274	2002721274	Ft. Detrick, then CDC	USA	1956	
275	NCTC 10245, GB11, 10399, China 5, 2002721275	CDC	China	1956	Lung and nose of horse
276	2002721276, G-2(3)	Naval Biological Lab	Canada/USA	1956	
277	2002721277, Kweiyang #4	Gleiser Army Med Serv Grad School	USA	1956	
278	2002721278, 6317440		NM/USA	1964	Human
279	2002721279, A193		NY/USA	1964	Human; from cord blood, nose, throat
304	2000031304, 2000031281, H1533	CDC	MD/USA	2000	Lab infection (human)

Ribotyping Approach

- **Size-fractionated *Pst*1 and *Eco*R1 fragments Southern transferred and probed with ^{32}P-labeled 18-mer derived from *E. coli* rDNA (gct cct agt acg aga gga)**

- **Objective is to identify restriction fragment length polymorphisms (RFLPs) from the relatively polymorphic regions flanking the rRNA operons**

- **Digests of strains not discriminated by ribotyping were also probed with two repeat sequences identified by the Tandem Repeats Finder program version 3.21 ("Repeat32" - ggc gtt tgg cgt ttg cgc ttt and "Repeat35" – tcg gcg gtt cgg cgt tcg gcg gt) using preliminary sequence data obtained from The Institute for Genomic Research through the website at http://www.tigr.org.**

Ribotyping Results

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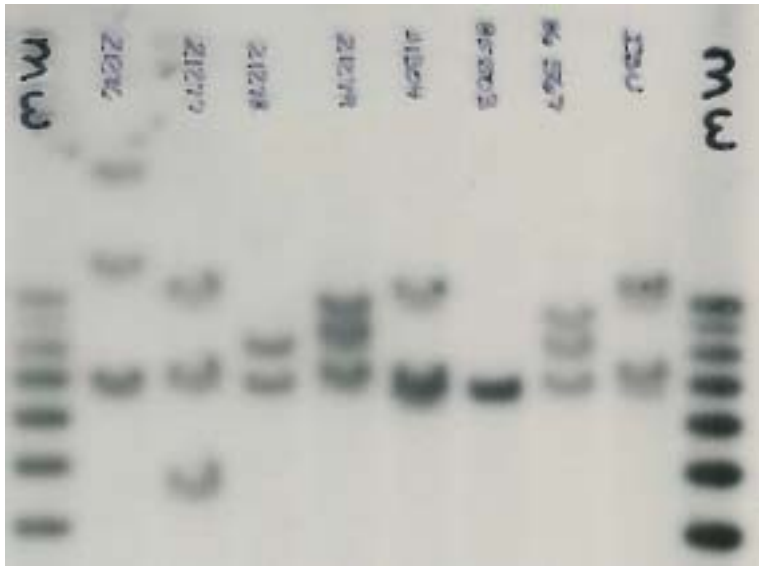
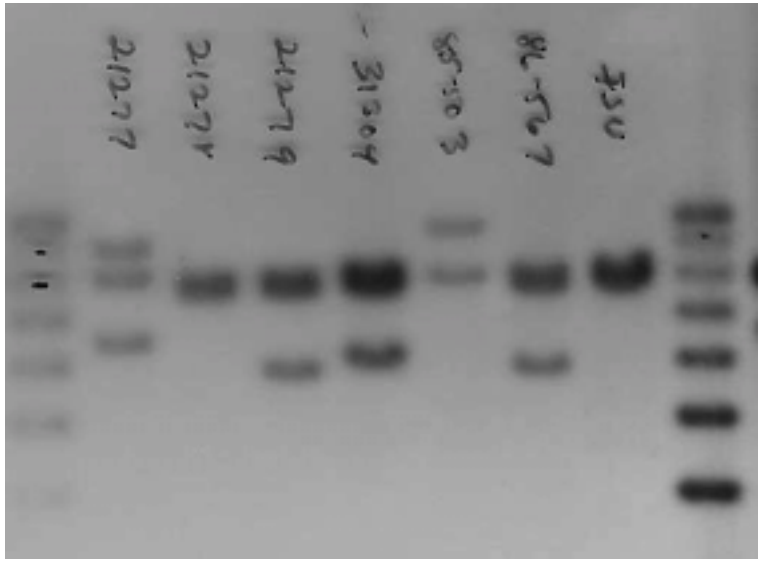
- **25 isolates resolved into 17 distinct ribotypes. Two of the 25 (GB8 and 31304) were known to be clonal and exhibited identical patterns, as expected.**



*Eco*R1 Digest/ rRNA Probe



*Pst*1 Digest/ rRNA Probe

Overall Genotyping Results

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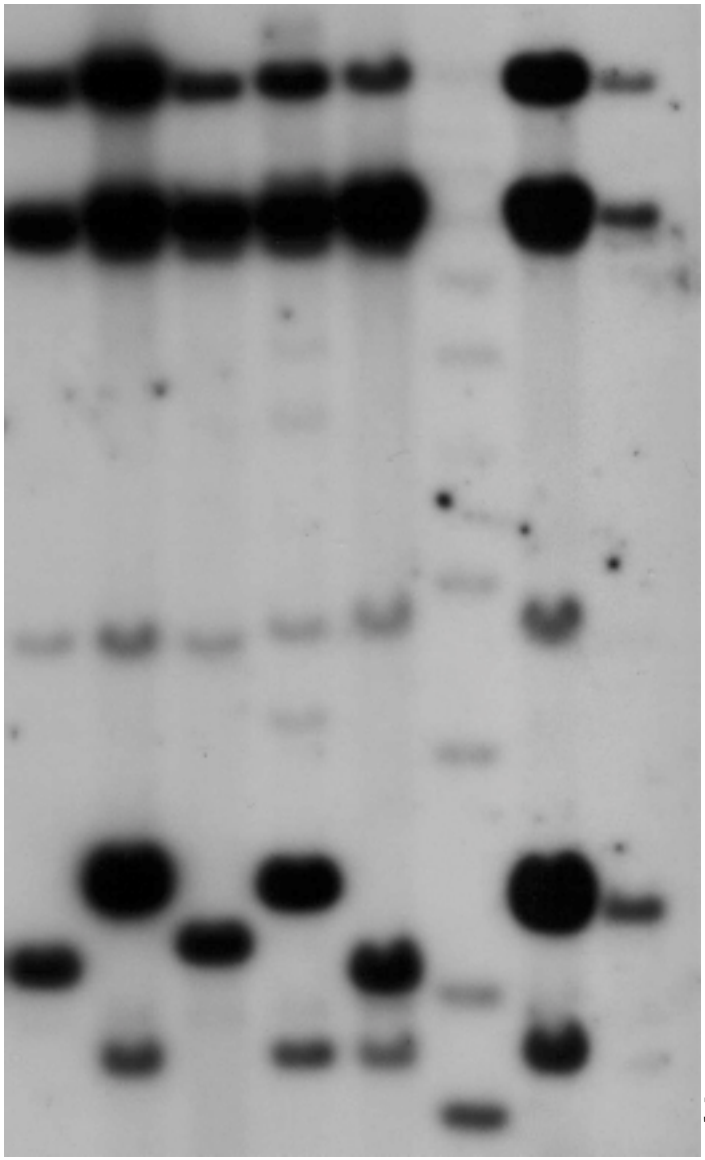
Probing with the two repeat sequences subsequently discriminated six others, so that a total of 22 genotypes were distinctly identified from 25 isolates (two of which were known to be clonal).



Observed rDNA Band Sizes (kb)

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Eco R1 Digests with rDNA Probe

	GB3	GB4	GB5	GB6	GB7	GB8	GB9	GB10	GB12	T2	T4	T6	T7	T9	273	274	275	276	277	278	279	304	503	567	ISU
~25																		~25							
~17							~17																		
~15								~15										~15							
~14							~14																		
12.0						12.0			12.0						12.0	12.0	12.0		12.0			12.0			12
11.2			11.2	11.2	11.2			11.2																	
10.2			10.2	10.2	10.2																10.2			10.2	
10.1		10.1								10.1	10.1	10.1	10.1	10.1						10.1					
9.2		9.2																							
9.0			9.0	9.0																					
8.8						8.8	8.8		8.8	8.8	8.8	8.8	8.8	8.8	8.8	8.8	8.8	8.8	8.8	8.8	8.8	8.8	8.8	8.8	8.8
8.4	8.4	8.4				8.4				8.4	8.4	8.4	8.4	8.4	8.4	8.4						8.4	8.4		8.4
7.5					7.5																				
7.2	7.2																								
6.6									6.6								6.6		6.6						
Designation	E-1	E-2	E-3	E-3	E-4	E-5	E-6	E-7	E-8	E-9	E-9	E-9	E-9	E-9	E-5	E-5	E-8	E-10	E-8	E-11	E-12	E-5	E-13	E-12	E-5

Pst 1 Digests with rDNA Probe

	GB3	GB4	GB5	GB6	GB7	GB8	GB9	GB10	GB12	T2	T4	T6	T7	T9	273	274	275	276	277	278	279	304	503	567	ISU
11.4							11.4							11.4									11.4		
10.9																			10.9						
10.1			10.1	10.1		10.1	10.1	10.1	10.1	10.1	10.1	10.1	10.1		10.1	10.1	10.1		10.1	10.1	10.1	10.1	10.1	10.1	10.1
10.0					10.0		10.0											10.0							
9.9		9.9	9.9	9.9		9.9			9.8	9.8	9.8	9.8	9.8	9.8	9.8	9.8	9.8	9.8		9.8	9.8	9.8		9.8	9.8
9.7																									
9.2	9.2				9.2			9.2																	
8.6	8.6	8.6																							
8.4					8.5														8.4						
8.1	8.1	8.1				8.1				8.1	8.1	8.1	8.1		8.1	8.1					8.1	8.1			
8.0			8.0	8.0										8.0				8.0						8.0	
Designation	P-1	P-2	P-3	P-3	P-4	P-5	P-6	P-7	P-8	P-5	P-5	P-5	P-5	P-9	P-5	P-5	P-8	P-10	P-11	P-8	P-5	P-5	P-12	P-3	P-8

Observed Bands – Pst1/Repeat32 Probe

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B. mallei Isolate #

Band Size (kb)

	GB3	GB4	GB5	GB6	GB7	GB8	GB9	GB10	GB12	T2	T4	T6	T7	T9	273	274	275	276	277	278	279	304	503	567	ISU
~14												~14												~14	
12.2	12.2	12.2	12.2	12.2	12.2	12.2	12.2	12.2	12.2	12.2	12.2	12.2	12.2	12.2	12.2	12.2	12.2	12.2	12.2	12.2	12.2	12.2	12.2	12.2	12.2
10.2	10.2	10.2			10.2		10.2																		
9.8										9.8		9.8	9.8				9.8	9.8		9.8	9.8		9.8	9.8	9.8
8.9		8.9										8.9					8.9							8.9	
8.2	8.2	8.2	8.2	8.2	8.2	8.2	8.2	8.2	8.2	8.2	8.2	8.2	8.2	8.2	8.2	8.2	8.2	8.2	8.2	8.2	8.2	8.2	8.2	8.2	8.2
7.9	7.9	7.9	7.9			7.9	7.9	7.9	7.9	7.9	7.9	7.9	7.9	7.9	7.9	7.9	7.9	7.9	7.9	7.9	7.9	7.9	7.9	7.9	7.9
6.2																								6.2	
5.9												5.9													
5.4																								5.4	
5.2				5.2	5.2																				
5.1		5.1										5.1													
4.7					4.7																				
4.3					4.3																				
3.8	3.8				3.8		3.8	3.8																	
3.7		3.7	3.7	3.7		3.7			3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7
3.2				3.2	3.2																			3.2	
3.0		3.0			3.0		3.0					3.0						3.0		3.0	3.0				
2.9																							2.9		
2.8										2.8								2.8		2.8	2.8			2.8	
2.6													2.6												
2.4				2.4		2.4		2.4	2.4						2.4	2.4	2.4		2.4			2.4		2.4	
2.3					2.3		2.3			2.3		2.3	2.3					2.3					2.3		
2.2	2.2	2.2	2.2																				2.2		
2.1											2.1			2.1						2.1	2.1				2.1
1,8	1.8	1.8		1.8	1.8	1.8			1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8
1.7	1.7	1.7					1.7	1.7									1.7	1.7	1.7					1.7	1.7
1.6					1.6					1.6	1.6	1.6								1.6			1.6		

Observed Bands – EcoR1/Repeat32

Edgewood Chemical Biological Center

B. mallei Isolate #

Band Size (kb)

	GB3	GB4	GB5	GB6	GB7	GB8	GB9	GB10	GB12	T2	T4	T6	T7	T9	273	274	275	276	277	278	279	304	503	567	ISU
~23	~23	~23	~23	~23	~23	~23	~23	~23	~23	~23	~23	~23	~23	~23	~23	~23	~23	~23	~23	~23	~23	~23	~23	~23	~23
~18	~18	~18	~18	~18		~18	~18		~18						~18	~18	~18	~18	~18			~18		~18	~18
~16	~16	~16		~16		~16	~16		~16						~16	~16						~16			
~13.5	~13.5	~13.5					~13.5																		
11.0										11.0															
9.8	9.8	9.8	9.8	9.8	9.8	9.8	9.8	9.8	9.8	9.8	9.8	9.8	9.8	9.8	9.8	9.8	9.8	9.8	9.8	9.8	9.8	9.8	9.8	9.8	9.8
7.6				7.6		7.6			7.6						7.6	7.6	7.6	7.6	7.6			7.6		7.6	
7.4	7.4	7.4	7.4		7.4		7.4	7.4		7.4	7.4	7.4	7.4	7.4						7.4	7.4		7.4		7.4
6.1				6.1																					
5.9		5.9	5.9		5.9		5.9	5.9		5.9	5.9	5.9	5.9	5.9						5.9	5.9				
5.8	5.8																5.8	5.8	5.8						
5.6						5.6			5.6							5.6						5.6	5.6	5.6	5.6
5.4	5.4	5.4	5.4	5.4	5.4	5.4	5.4	5.4		5.4	5.4	5.4	5.4	5.4	5.4					5.4	5.4				

Observed Bands – Pst1/Repeat35

Edgewood Chemical Biological Center

B. mallei Isolate

Band Size (kb)

	GB3	GB4	GB5	GB6	GB7	GB8	GB9	GB10	GB12	T2	T4	T6	T7	T9	273	274	275	276	277	278	279	304	503	567	ISU
~18												~18													~18
~14												~14			~14	~14		~14	~14	~14		~14		~14	~14
12.0						12.0				12.0	12.0		12.0	12.0	12.0	12.0		12.0	12.0	12.0		12.0	12.0	12.0	12
10.0	10.0	10.0	10.0	10.0	10.0	10.0			10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10
9.8												9.8							9.8						
9.0		9.0		9.0																					
8.5										8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5
8.2				8.2					8.2																
7.6										7.6	7.6	7.6	7.6		7.6	7.6	7.6		7.6	7.6	7.6	7.6	7.6	7.6	7.6
7.2			7.2	7.2		7.2			7.2		7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2		7.2
5.9												5.9													
5.1												5.1												5.1	
4.6																								4.6	
4.3				4.3	4.3			4.3		4.3	4.3	4.3			4.3			4.3					4.3	4.3	
4.2		4.2	4.2							4.2	4.2	4.2			4.2					4.2					
4.1									4.1																
4.0	4.0					4.0				4.0	4.0			4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0			4
3.6	3.6	3.6	3.6	3.6	3.6	3.6		3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6
3.4									3.4																
3.2			3.2	3.2				3.2		3.2	3.2	3.2	3.2	3.2			3.2	3.2	3.2	3.2					3.2
3.0																					3.0		3.0	3.0	
2.9	2.9	2.9	2.9	2.9	2.9							2.9	2.9		2.9									2.9	
2.4				2.4	2.4	2.4		2.4	2.4						2.4	2.4	2.4		2.4			2.4		2.4	
2.3																								2.3	
2.2	2.2	2.2	2.2							2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2		2.2	2.2	2.2	2.2	2.2	2.2
2.1											2.1			2.1						2.1	2.1				2.1
1.9	1.9	1.9			1.9					1.9	1.9					1.9	1.9		1.9	1.9	1.9	1.9	1.9	1.9	1.9
1.8										1.8	1.8	1.8	1.8			1.8		1.8	1.8			1.8		1.8	1.8
1.7																			1.7						
1.5										1.5		1.5													
1.4															1.4	1.4						1.4			
1.3	1.3																1.3	1.3	1.3		1.3		1.3	1.3	1.3
1.2		1.2						1.2									1.2								

Observed Bands – EcoR1/Repeat35

Edgewood Chemical Biological Center

B. mallei Isolate

Band Size (kb)

	GB3	GB4	GB5	GB6	GB7	GB8	GB9	GB10	GB12	T2	T4	T6	T7	T9	273	274	275	276	277	278	279	304	503	567	ISU
~23	?		~23	~23	~23	~23			~23		~23	~23	~23	~23	~23		~23		~23	~23	~23	~23			~23
~18	?	~18	~18	~18	~18	~18			~18	~18	~18	~18	~18	~18	~18	~18	~18	~18	~18	~18	~18	~18	~18	~18	~18
~16				~16																					
~14		~14											~14	~14			~14		~14						~14
~13		~13	~13	~13	~13	~13	~13	~13	~13	~13	~13	~13	~13	~13	~13	~13	~13	~13	~13	~13	~13	~13	~13	~13	
11.5															11.5										
10.5				10.5	10.5	10.5			10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10..5	10.5	10.5	10.5	10.5	10.5	10.5
10.0					10.0		10.0	10.0						10.0	10.0		10.0				10.0				
9.8													9.8												
9.4													9.4												
8.8			8.8		8.8					8.8	8.8	8.8	8.8								8.8			8.8	
7.6				7.6		7.6			7.6						7.6	7.6	7.6	7.6	7.6			7.6			
7.3	7.3	7.3	7.3		7.3		7.3	7.3		7.3		7.3	7.3											7.3	
7.2											7.2			7.2						7.2	7.2		7.2		7.2
7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0
6.2			6.2	6.2		6.2																			
6.1								6.1	6.1	6.1	6.1	6.1	6.1	6.1	6.1	6.1	6.1	6.1	6.1	6.1	6.1	6.1	6.1	6.1	6.1
6.0		6.0			6.0																				
5.9		5.9					5.9											5.9							
5.7														5.7	5.7	5.7	5.7		5.7						
5.3		5.3	5.3	5.3	5.3	5.3	5.3	5.3	5.3	5.3	5.3	5.3	5.3	5.3	5.3	5.3	5.3	5.3	5.3	5.3	5.3	5.3	5.3	5.3	5.3
5.2															5.2		5.2								
5.0													5.0		5.2										
4.9							4.9	4.9																	
4.5						4.5	4.5									4.5						4.5			
4.4																		4.4							4.4
4.3		4.3		4.3	4.3		4.3		4.3								4.3		4.3						
4.2			4.2			4.2		4.2	4.2	4.2	4.2	4.2	4.2	4.2	4.2		4.2		4.2	4.2					4.2
4.1	4.1							4.1			4.1			4.1	4.1	4.1		4.1		4.1	4.1	4.1	4.1	4.1	
4.0			4.0	4.0																					
3.2			3.2	3.2											3.2										

Pairwise Discrimination of 25 Isolates

Edgewood Chemical Biological Center

E = *Eco* R1 ribotyping | P = *Pst* 1 ribotyping | E32 = Repeat32 probe of *Eco* R1 digests | P35 = Repeat35 probe of *Pst* 1 digests

E,P = Both *Eco* R1 and *Pst* 1 ribotyping | P32 = Repeat32 probe of *Pst* 1 digests | E35 = Repeat35 probe of *Eco* R1 digests

Strain	GB4	GB5	GB6	GB7	GB8	GB9	GB10	GB12	T2	T4	T6	T7	T9	273	274	275	276	277	278	279	31304	85-503	86-567	ISU
GB3	E,P	E,P	E,P	E,P	E,P	E,P	E,P	P	E,P	E,P	E,P	E,P	E,P	E,P	E,P	E,P	E,P	E,P	E,P	E,P	E,P	E,P	E,P	E,P
GB4	------	E,P	E,P	E,P	E,P	E,P	E,P	P	E,P	E,P	E,P	E,P	E,P	E,P	E,P	E,P	E,P	E,P	E,P	E,P	E,P	E,P	E,P	E,P
GB5		------	P32 E32 P35 E35	E,P	E	E,P	E,P	P	E	E	E	E	E,P	E	E	E,P	E	E,P	E,P	E	E	E,P	E	E,P
GB6			----------	E,P	E	E,P	E,P	P	E	E	E	E	E,P	E	E	E,P	E	E,P	E,P	E	E	E,P	E	E,P
GB7				-------	E,P	E,P	E,P	P	E,P	E,P	E,P	E,P	E,P	E,P	E,P	E,P	E,P	E,P	E,P	E,P	E,P	E,P	E,P	E,P
GB8					-------	E,P	E,P	P	E	E	E	E	E,P	E35 P35 (only)	None	E,P	E	E,P	E,P	E	None	E,P	E	P
GB9						-------	E,P	P	E,P	E,P	E,P	E,P	E,P	E,P	E,P	E,P	E,P	E,P	E,P	E,P	E,P	E,P	E,P	E,P
GB10							--------	P	E,P	E,P	E,P	E,P	E,P	E,P	E,P	E,P	E,P	E,P	E,P	E,P	E,P	E,P	E,P	E,P
GB12								-------	P	P	P	P	P	P	P	None	P	P	E	P	P	P	P	E
T2									-----	P32 E32 P35 E35	P32 E32 P35 E35	E32, P32 only	P	E	E	E,P	E	E,P	E,P	E	E	E,P	E	E,P
T4										----------	P32 E35 P35 only	P32 E35 P35 only	P	E	E	E,P	E	E,P	E,P	E	E	E,P	E	E,P
T6											----------	P32 E35 P35 only	P	E	E	E,P	E	E,P	E,P	E	E	E,P	E	E,P
T7												----------	P	E	E	E,P	E	E,P	E,P	E	E	E,P	E	E,P
T9													------	E,P	E,P	E,P	E,P	E,P	E,P	E,P	E,P	E,P	E,P	E,P
273														----------	E35, P35 only	P	E	E,P	E,P	E	E35 P35 only	E,P	E	P
274															----------	P	E	E,P	E,P	E	None	E,P	E	P
275																-------	E,P	P	E	E,P	E,P	E,P	E	E
276																	-------	E,P	E,P	E	E	E,P	E	E,P
277																		------	E,P	E,P	E,P	E,P	E,P	E,P
278																			-------	E,P	E	E,P	E,P	E
279																				------	E	E,P	P32 E32 P35 E35	E,P
31304																					----------	E,P	E	P
85-503																						--------	E,P	E,P
86-567																							----------	E,P
ISU																								------

Genotyping Scheme – How to discriminate isolates

Edgewood Chemical Biological Center

25 *B. mallei* Isolates

Eco R1 and *Pst* 1 Digests probed with rDNA sequence

Step 1. Ribotyping

17 Different Ribotypes:

Ribotype:	E-1/P-1	E-2/P-2	E-3/P-3	E-4/P-4	E-5/P-5	E-6/P-6	E-7/P-7	E-8/P-8	E-9/P-5	E-9/P-9	E-10/P-10	E-8/P-11	E-11/P-8	E-12/P-5	E-13/P-12	E-12/P-3	E-5/P-8
Isolate(s):	GB3	GB4	GB5 GB6	GB7	GB8 273 274 304	GB9	GB10	GB12 275	T2 T4 T6 T7	T9	276	277	278	279	503	567	ISU

Eco R1 or *Pst* 1 Digests probed with either Repeat 32 or Repeat35

Eco R1 or Pst1 Digests probed with Repeat35

Indistinguishable

Pst 1 digest probed with Repeat32

Step 2. Repeat32 and Repeat35 sequence probing

GB5	GB6	GB8 274 304	273	T2	T4	T6	T7

Indistinguishable

(GB8 and 304 are known to be clonal)

Result: 22 different genotypes from 25 isolates (each red box represents one unique genotype)

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